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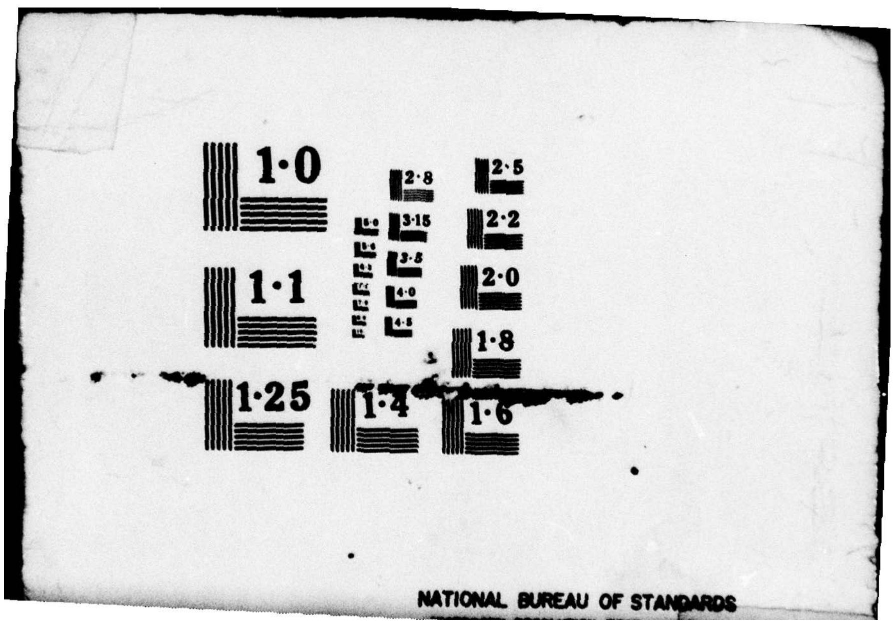
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BODY COMPOSITION IN EXPERIMENTAL HUMAN SCURVY: A PARTIAL STUDY

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HUMAN SUBJECTS PARTICIPATED IN THESE STUDIES AFTER GIVING THEIR FREE AND INFORMED CONSENT.

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Experimental scurvy was induced in five adult males, 26 to 52 years of age. Body compartment measurements during recovery were derived from body density by water displacement, total body water from deuterium dilution and total body potassium from whole body counting of ^{40}K. The first body composition measurements were accomplished after measured amounts of vitamin C had been administered daily for 18, 24 or 31 days. A second measurement was made approximately 17 weeks later. Attempts to maintain constant body weight failed. The subjects

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gained a mean of 0.2 kg during depletion. Between early and late repletion they gained a mean of 4.76 kg. Body fat exhibited the greatest mean body compartmental change followed by small mean changes in the body water or dry protein compartments. Correlations during early repletion between body pool, plasma or whole blood ascorbate with the dry protein mass (based on three independent techniques of determining this compartment) were of moderate inverse significance. All of the techniques used to estimate the size of the dry protein compartment did not detect evidence of impaired protein utilization or correction of protein utilization previously impaired by vitamin depletion during recovery from vitamin C deficiency. However nitrogen balance data from this study did not disclose the impairment in protein utilization suggested by our initial study on experimental scurvy.

ABSTRACT

Experimental scurvy was induced in five adult males, 26 to 52 years of age. Body compartment measurements during recovery were derived from body density by water displacement, total body water from deuterium dilution and total body potassium from whole body counting of ^{40}K. The first body composition measurements were accomplished after measured amounts of vitamin C had been administered daily for 18, 24 or 31 days. A second measurement was made approximately 17 weeks later. Attempts to maintain constant body weight failed. The subjects gained a mean of 0.2 kg during depletion. Between early and late repletion they gained a mean of 4.76 kg. Body fat exhibited the greatest mean body compartmental change followed by small mean changes in the body water or dry protein compartments. Correlations during early repletion between body pool, plasma or whole blood ascorbate with the dry protein mass (based on three independent techniques of determining this compartment) were of moderate inverse significance. All of the techniques used to estimate the size of the dry protein compartment did not detect evidence of impaired protein utilization or correction of protein utilization previously impaired by vitamin depletion during recovery from vitamin C deficiency. However nitrogen balance data from this study did not disclose the impairment in protein utilization suggested by our initial study on experimental scurvy.

FOREWORD

In the period 1966 to 1968, two closely controlled studies (coded Scurvy I and Scurvy II) to determine the adult human requirements for vitamin C were conducted at the University Hospital, Iowa City in close colloboration between the Department of the Internal Medicine, University of Iowa, and the US Army Medical Research and Nutrition Laboratory (USAMRNL), Denver, Colorado. Scurvy I involved four volunteers and covered the period of early November 1966 to late spring 1967. Initital analysis of the data revealed that more subjects were necessary to define adequately the requirement for the vitamin and to study some of the physiological problems encountered. The preliminary phases of Scurvy II were begun in October 1967. While the data analysis and interpretation of the major aspects of Scurvy I had been completed prior to completion of the protocol for Scurvy II, analysis of some data elements of Scurvy I had not been completed.

In the third reference to this report, Baker et al described a method of estimating the total body pool of ascorbic acid in young adult human males and measured the rate of utilization of the vitamin. Using body density techniques, body fat had been determined and, by inference, fat free body weight. These investigators demonstrated that both the pool size and the rate of utilization of the vitamin in healthy young males were directly correlated with the fat free body compartment. On analysis of data derived during Scurvy I (Reference 2) an abnormality in nitrogen metabolism during depletion of the vitamin was noted with slow but complete correction of this abnormality during the repletion phase. It was felt that the magnitude of this abnormality should be reflected as a decrease followed by an increase in the dry protein compartment of the fat free mass during depletion/repletion respectively. Body composition had not been studied during C deficiency in the human. The addition of body composition studies to Scurvy II appeared appropriate. At the time of these studies, a most unusual and well staffed whole body counting facility (Reference 12) was located at the Veterans Administration Hospital, Iowa City. It was hoped that with use of the body volumeter from USAMRNL, the whole body counter at the VA Hospital, Iowa City, and other techniques and experience of personnel of the Bioenergetics Division, USAMRNL, that a fairly clear picture of body composition changes due to scurvy could be obtained. Drs. Hodges and Hood obtained the cooperation of Dr. Richard E. Peterson, Chief of the Whole Body Counting Facility.

It was unfortunate that the data analysis that pinpointed the need for measurement of body composition had not been completed prior to initiation of Scurvy II, because "before" studies were not obtained. Due to the extremely tight schedule of the subjects, during the final portion of the depletion phase and the early portion of repletion, it was impossible to conduct the measurements described in this report until the repletion phase had well begun. This prevented a total evaluation of the impact of vitamin C deficiency upon the body composition of our volunteers.

Due to the current philosophy pertaining to human experimentation, it would now be impossible to conduct a similar study. Therefore, we are publishing our findings in this form. We recognize that the total study is incomplete. The same subject identification codes have been used in this report as were used in previous publications from these studies to permit cross reference.

This manuscript was completed in the early 1970s and forwarded to the Scientific Publication Review Committee in 1973 for clearance. Unfortunately due to unforseen circumstances, the manuscript was lost from processing but was found while the senior author was working as a special consultant on another project at LAIR in December 1978.

Since this publication was initially submitted Mr. Harry Krzywicki has retired from Federal Civil Service, and COL E. M. Baker has retired from the Army. Both reside in Aurora, Colorado. Dr. James Hood lives in Cedar Rapids, Iowa, where he is in the practice of Internal Medicine.

A word of appreciation for the strong support provided by the following individuals is indicated: Marie Paule Yvette Rogers, Kay Robson, and Wanda Zweigle. Mr. Bruce Schwenneker is to be thanked for his reworking of the statistical routines and data that were needed for final evaluation of the material. Gratitude is expressed to Dr. Peterson and his staff at the VA Hospital, Iowa City, Iowa, for the contribution they made to this study.

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INTRODUCTION

In a recent metabolic study (1,2) of experimental scurvy, the urinary nitrogen-creatinine ratios indicated that negative nitrogen balances occurred during vitamin C depletion despite daily intakes of 90 g of protein in a 3,000 calorie diet, adequate in all other essential nutrients. This suggested that protein utilization was impaired and the loss of body protein appears probable during ascorbic acid deprivation. During vitamin C repletion, these negative nitrogen balances were reversed. The relationship of the ascorbic acid pool size and the metabolism of the vitamins to the fat free body mass was only briefly noted by Baker et al (3). However, in neither study had there been any attempt made to estimate body composition in terms of the major components, namely, water, fat, protein and minerals.

During the second study of vitamin C deprivation in man (4), it was proposed that body composition measurements be performed in an attempt to relate blood and plasma levels, or pool size of ascorbic acid to various body compartments. The body composition changes and vitamin C levels will be presented in this paper.

METHODS

Five male subjects, ranging between 26 and 52 years of age (mean age 36 years) were fed a normal control diet containing soy protein products with 2.5 mg of ascorbic acid plus a 75.0 mg supplement of the vitamin daily for 13 days. They were then fed a liquid formula diet free of ascorbic acid for periods ranging from 84 to 97 days when obvious clinical signs of scurvy ranging from petechial hemorrhages to edema (4) had appeared. Repletion consisted of graded quantities of ascorbic acid taken by mouth daily. Body composition measurements were performed on day 129 of the study. At this time two subjects had already been repleted with vitamin C for 31 days. Two others were in day 24 of repletion, and the one remaining subject had been repleted for 18 days. However, some clinical signs of scurvy (hyperkeratosis, conjunctival lesions, swelling of the groin) still existed at this time. After the subjects had been repleted for approximately 17, 18 or 20 weeks, body compartments were again studied. The metabolic aspects of this study were reported by Baker et al (5). The methods for measuring body composition have been previously described by this laboratory (6-9) and

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1. Hodges, R.E., et al. Am J Clin Nutr 22:535-548, 1969.
2. Baker, E.M., et al. Am J Clin Nutr 22:549-558, 1969.
3. Baker, E.M., et al. Proc Soc Exptl Biol Med 109:737-741, 1962.
4. Hodges, R.E., et al. Am J Clin Nutr 24:432-443, 1971.
5. Baker, E.M., et al. Am J Clin Nutr 24:444-454, 1971.
6. Krzywicki, H.J., et al. Am J Clin Nutr 21:87-97, 1968.
7. Allen, T.H., et al. J Appl Physiol 14:1005-1008, 1959.
8. Allen, T.H., et al. J Appl Physiol 14:1009-1012, 1959.
9. Allen, T.H., et al. USAMRNL Report No. 250, Sep 1960.

include total body fat, water, dry protein and mineral estimates as derived from body density, and total body water as calculated from deuterium oxide dilution (10). Body potassium (11) was measured in a whole body potassium-40 counter at the Veterans Hospital, Iowa City, Iowa (12). At the time of the first whole body counting four of the five volunteers agreed to take ^{42}K for calibration purposes and upon counting in late repletion three agreed to a repeat ^{42}K calibration measurement. The 5 microcurie ^{42}K dose provided a measurement factor of "efficiency of detection calibration." When a subject declined to take the ^{42}K dose the calibration factor was derived from data obtained from other volunteers of similar age, height and weight.

Several anthropometric measurements were taken and included extremity and trunk girths, and the arm and scapula skinfolds. The relationship of the protein compartment to ascorbic acid pools and concentrations was statistically analyzed by the technique of Dunn (13) and body compartments were subjected to an analysis of variance (14) for the five subjects for one repeated measure.

RESULTS

Table 1 describes the mean observed and derived values of various body compartments for the five subjects after 18 to 31 days and 17 to 20 weeks of vitamin C repletion. The data include the mean body density, body weight, and the calculated values for total body water, fat, dry protein, and mineral. The observed values for total body water determined by deuterium oxide (D_2O) dilution are also included.

The mean body density had been 1.050 g/ml after 18 to 31 days of repletion and then significantly decreased to 1.038 g/ml after 17 to 20 weeks of repletion. This indicates increased body fat. Body weight increased from a mean of 72.34 kg in early repletion to 77.10 kg, representing a 4.76 kg (6.58% of body weight) gain. The total body water compartment was insignificantly decreased 0.09 kg or a 0.22% loss, however this reflected a 3.5% decrease of body water when expressed as percent of body weight. Body fat was increased by 4.89 kg which reflected a 27.84% gain in this compartment, equal to a 4.8% increase when considered as percent body weight. The insignificant 0.03 kg decrease in dry protein was only a 0.27% loss in this body compartment which was the same percent loss as that noted for minerals (0.01 kg or 0.27%) and reflected only minor, decreases as percent body weight.

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10. Nielsen, W.C., et al. J Appl Physiol 31:957-961, 1971.
11. Allen, T.H., et al. J Gerontol 15:348-353, 1960.
12. Peterson, R.E., et al. In: Radioactivity in Man, edited by Meneely and Linde. Springfield, C.C. Thomas, 1965, pp 71-86.
13. Dunn, O.I. J Am Stat Assn 54:613-621, 1959.
14. Snedecor, G.W. and W.G. Cochran. Statistical Methods, 6th ed. Iowa State College Press, Ames, 1967.

The mean body water values as determined by D_2O dilution showed an increase of 0.44 kg or 1.01% in the water compartment after repletion. These body water values were approximately 3-4 kg higher than those calculated from body density. The values obtained by D_2O dilution represent 79.4 and 80.4% of the fat free mass.

Individual data for each of the five subjects are presented in Table 2 as a matter of interest and depicts changes that occurred in the various body compartments during vitamin C repletion. Subject M lost 1.06 kg of body weight during repletion, while subject R gained but 0.34 kg in contrast to gains averaging approximately 8 kg in the other three subjects. The mean changes represented a gain of 4.76 kg of body weight and reflect the inconstancy of dietary intake among the subjects.

Table 3 cites the individual and mean values of the dry protein compartment as calculated (a) from total body water from D_2O dilution (assuming that body water constitutes 73.2% of the fat free mass and 20.2% is dry protein); (b) similarly from body density estimates of the fat free mass, and (c) from whole body radioisotope potassium counting (providing a 4.5% correction is made for potassium that has been measured but which is present as a mineral in the body mineral compartment). The mean value calculated from body water in early repletion was approximately 9.0% higher than that calculated from body density. The mean values obtained by ^{40}K counting were lower than those derived from body water. This same trend was also noted during late vitamin C repletion at which time the dry protein mass (12.13 kg) derived from body water was now about 10% higher than the body density values, but only the values derived from body water exceeded the ^{40}K values. The dry protein compartment as measured by ^{40}K was significantly increased during repletion but not as estimated by the other two techniques.

Total estimated ascorbic acid levels in the body, and the concentration in plasma and whole blood are shown in Table 4. The body pool and plasma levels appear to reflect the intake as noted in subject M, who had been repleted on an average daily intake of 129.5 mg for 31 days. Subjects S and R followed, S having been rehabilitated on 1,229.5 mg over 31 days (average 39.7 mg/day) while R had received 1,197 mg over 18 days (average 66.5 mg/day). Subject H had the lowest titers of body pool and plasma vitamin C since his intake totaled only 134.5 mg over 24 days (average 5.6 mg/day). Whole blood levels of ascorbate were more varied than the plasma levels. Individual values for body pool levels were not available on day 247 of the study (late repletion) except to estimate that they exceeded 1,500 mg. At this time the blood plasma and whole blood ascorbate levels were slightly above normal (normal blood plasma vitamin C levels range from 0.4 to 1.2 mg/dl and whole blood levels range from 0.5 to 1.6 mg/dl).

Table 5 describes the correlation coefficients obtained between the body ascorbic acid pool, whole blood, and plasma ascorbic acid

levels as related to body fat and the dry protein compartments as calculated from D_2O dilution, body density and whole body counting of potassium. No definite trends were noted during early repletion, however the dry protein mass of the body demonstrated higher correlates (although negatively) with the ascorbate spaces than did the body fat compartment. In two instances, the body fat compartment as calculated from ^{40}K counting correlated well with body pool and plasma ascorbic acid (r=-0.831 and -0.786, respectively). An inverse relationship was observed in all correlates of ascorbic acid spaces to the dry protein mass. With long term repletion, there was even less correlation between the body compartments and the parameters reflecting ascorbate status.

Table 6 depicts various anthropometric measures accomplished during early repletion and after long term repletion with vitamin C. The changes in body diameters and circumferences ranged from a low of 1.3% to a high of 6.7%. However, the skinfold thicknesses decreased considerably reflecting 8 to 33% losses. Statistical analysis indicated, however, that only the calf circumference showed any significant increase based on an analysis of confidence intervals for mean differences (13). This was further corroborated by the paired-t test. No other significant changes in the anthropometric observations were noted upon repletion of the subjects. As could be expected the subjects showing the greatest increases in body and extremity circumferences and body diameters were those demonstrating the greatest gain in body weight.

In this study the plots of the daily urinary nitrogen, creatinine and nitrogen-creatinine ratio for the average man were either flat or not indicative of an impairment in proteins utilization as suggested in the earlier study (1). Hence this study on body composition would have difficulty in detecting changes in protein utilization.

DISCUSSION

Hodges et al (1) attempted to relate the appearance and severity of the clinical signs of scurvy to the body pool size of vitamin C and rate at which the body pool was depleted during complete dietary deprivation of the vitamin. Although typical signs of clinical scurvy were manifest, many physiological functions such as electroencephalograms, basal metabolic rates, various blood parameters and wound healing remained essentially normal during deprivation of the vitamin. Urinary nitrogen and creatinine excretion ratios, however, suggested that negative nitrogen balances occurred during depletion and balances then became positive with repletion. A repletion dose of 10.5 to 66.5 mg of ascorbic acid per day and in one instance, a low intake of 6.5 mg/day appeared adequate to cure scurvy. When a saturation dose of 600 mg of unlabelled vitamin C was fed during the final two weeks of the study, the urinary excretion of reduced ascorbic acid served only to reflect the high intake.

Despite attempts to minimize body weight loss by adjusting caloric intake there was considerable fluctuation throughout the study. The

mean weight gain of 4.76 kg during repletion reflected a range of from a 1.06 kg loss in one subject to a gain of 8.32 kg in another. It is obvious that the gain in body weight could be attributed mainly to body fat which is the most variable body component. This was demonstrated in three of the five subjects. Hodges et al (1) reported that isocaloric manipulation of dietary ingredients was necessary during the repletion phase of the study to insure the maintenance of adequate protein intake since some of the subjects rejected the soy protein diet. The subjects were to have exercised to maintain appetite and energy expenditure. Apparently some of the men consumed calories in excess of their expenditure.

The changes in body water were nominal for all but two subjects, M and R. Subject M had lost 1.06 kg of body weight and was calculated to have lost 1.75 kg of body water, 0.48 kg of protein and 0.16 kg of mineral while he was calculated to have gained 1.34 kg of body fat. This subject was moderately scorbutic at the end of the depletion period. Although his appearance did not suggest edema at the time of our first measurement, some body water may have been retained at that time. The same may be said for subject R, whose water loss of 0.51 kg after repletion reflected only a 3.5% loss of water when expressed as percent of body weight. Subject H, who exhibited the most prominent signs and symptoms of scurvy, had developed the greatest degree of edema, but all clinical signs of edema had disappeared at the time of the first measurement which was actually on day 24 of repletion for subject H. However, scurvy is known to be associated with fluid retention. Subject H initially was under treated during initial repletion with only 4 mg of ascorbic acid/day. On increase of the supplement to 6.5 mg/day, he underwent a marked diuresis with weight loss of over eleven kilograms. This aspect of the study is well discussed by Hodges et al (4). Considering that H, who had the most severe scurvy and the lowest rate of vitamin repletion, had apparently completed diuresis by the time of these body composition measurements, it can probably be assumed that fluid retention was not a problem for the other four subjects when the first body composition measurements were made.

Table 7 presents the body weight of each volunteer for the end of the control period, the end of the vitamin C depletion period, at the time in early repletion of the initial body composition measurements and, finally, during the late repletion phase. It can be noted that during depletion three of the volunteers, H, M and R actually gained weight. While three of the subjects had exhibited edema by the end of the depletion period (4), it is possible that all had retained a certain amount of fluid. This is suggested by the weight loss that was demonstrated by all five of the subjects between the end of depletion and the time of the first body composition measurement.

The next compartment of consequence is the dry protein mass which was examined from three different aspects. The dry protein mass represents, essentially that portion of the body remaining after all water, fat and minerals have been removed. Namely, it contains muscle and

non-muscle protein with traces of carbohydrate. It is difficult to separate this mass in humans any further by applying factors gained from animal studies reported by Chinn (15), however it best represents the metabolizing mass of the body as can be defined from densitometry. Moore et al (16) described this mass when hydrated, as the oxygen-exchanging, potassium-rich, glucose-oxidizing, work performing tissue.

The size of this mass could be influenced by alterations in the estimation of body hydration. Forbes and Lewis (17) showed that water constituted 67.4 and 70.4 % of two cadavers they analyzed, while Behnke et al (18) reported that water made up 71.2% of the lean body mass (containing 2% essential fat). Yet, the most commonly used coefficient of body water, 73.2% of lean body mass, was developed by Pace and Rathbun (19) based upon multiple studies done by multiple investigators in multiple species including rats, dogs, pigs, monkeys, and others. If a smaller coefficient were to be used for example, 72% in an individual of 70 kg body weight with a 58.8 kg of lean body mass, a 705 g reduction of body water can be calculated which would then need to be accepted as either dry protein or mineral.

Densitometrically, alterations of body compartment estimates can be induced by ingestion of water prior to measurement, or conversely, measuring when subjects are hypohydrated. One liter of consumed water could increase the dry protein mass calculation by 280 g. It is felt that the densitometric estimate of body compartments is the most accurate since the authors (9) considered body hydration when they formulated (9) the equation.

Forbes and Lewis (17) in their studies concluded that 60 % of all body potassium is contained in muscle, and the counting of whole body potassium should reflect a reasonable estimate of the lean body mass based on their coefficient of 68.1 mEq of potassium per kilogram of this mass.

Table 3 shows the disparity in quantification of this compartment as it has been previously described (6). If the densitometric values are accepted as the most valid, it appears that the body water calculation of dry protein overestimates and the ^{40}K values underestimate this compartment. During the early repletion phase of the study, the mean values of the body water and ^{40}K estimates appear to reflect the densitometric calculation of the dry protein mass. By late repletion, the dry protein mass increased. This was noted in the body water estimates of the dry protein in four of the five subjects and in two

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15. Chinn, K.S.K., J Nutr 90:323-330, 1966.
16. Moore, F.D., et al. The Body Cell Mass and Its Supporting Environment. Philadelphia: W.B. Saunders Co., 1963.
17. Forbes, G.B. and A.M. Lewis. J Clin Invest 34:596-600, 1956.
18. Behnke, A.R., et al. Human Biol 31:213-234, 1959.
19. Pace, N. and E.N. Rathburn. J Biol Chem 158:685-691, 1945.

subjects by density estimate. It was anticipated that this compartment would specifically correlate with either body pool or blood and plasma levels of ascorbic acid.

The correlation coefficients showed that the dry protein mass in most instances was better related in an inverse manner to ascorbic acid, during the early repletion phase of the study with the exception that the body pool or plasma levels were inversely related to fat when derived from ^{40}K counting. No explanation is offered for the wholly unexpected high inverse correlates noted for plasma and body pool levels of vitamin C to body fat as derived from whole body potassium counting, except to say that perhaps they are biologically not significant. Certainly, with the variability of the body fat compartment which usually is increased with age, poor correlation with ascorbic acid levels would be expected (20).

The anthropometric data revealed no significant differences to exist between the body diameters or circumferences taken after early and long term repletion. This is probably due to the fact that three of the subjects gained weight, one remained unchanged and another lost weight. This resulted in a standard deviation too great to reflect any significance.

It can be said that under the conditions of this experiment, the greatest change was observed in the body fat compartment due to the fact that upon repletion, food intake was manipulated to maintain protein intake. With limited energy expenditure weight gain was to have been anticipated. The body water compartment exhibited some change during repletion but none that was attributable to the vitamin deficiency induced edema since repletion was well underway at the time that body composition measurements were made. None of the body composition techniques used were sensitive enough to demonstrate that protein utilization was perhaps impaired during this study as was suggested by Hodges et al (1) or as observed by Torre and Green (21) in animal experiments.

In 1962, Baker et al (3) reported on the estimation of the body pool size and utilization rate of ascorbic acid in normal adult males. Their volunteers had been subjected to body composition studies; the measurements were obtained with a body volumeter. The fat free mass of the volunteers was determined to be directly related to both the pool size of vitamin C and the utilization rate of the vitamin. Body pool size in the six volunteers in that study averaged 1,838 mg. During the study, the men were on a normal diet with a normal vitamin C intake.

Under the condition of the current study, it is difficult to relate utilization rate or body pool to the fat free mass. The difficulty

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20. Krzywicki, H.J. and K.C.K. Chinn. Am J Clin Nutr 20:305:310, 1967.
21. Torre, N.P. and F.A. Green. J Nutr 97:61-64, 1969.

lies in the fact that the subject's fat free mass had not been determined prior to the initiation of the vitamin free diet. The fat free mass determined in early repletion can only be considered as a reflection of the fat free mass taken 18 to 31 days into repletion with the subjects receiving varying levels of ascorbic acid and cannot be directly translated to the fat free mass at the end of depletion/beginning of repletion. Table 7 shows that the body weights obtained in late repletion were similar in three of the five subjects with those obtained prior to the depletion phase but total body weight can not be translated into fat free mass.

It is unfortunate that Baker et al (5) did not identify the specific size of the body pools of the individual subjects immediately prior to the deficiency phase of the study. They provided us with the individual body pool sizes on specific days of the depletion phase and provided regression curves which permit calculation of the approximate body pool size of the individuals prior to depletion. In Table 8 a comparison of the fat free mass is made with the body pool size and utilization rates of the vitamin. The mean estimated initial body pool is 1876 mg which is similar to the group mean reported in reference 3. The fat free mass for both early and late repletion is given. The rate of catabolism of the existing body pool, which is discussed in detail in reference 5, is a measure of utilization and with the exception of subject S, it appears to be related to the lean body mass. On the other hand, the estimated initial body pool also appears to be closely related to the fat free mass with the exception of subject M whose initial body pool appears to be larger than one would expect from previous observations. The correlation coefficient for the fat free mass versus the initial body pool indicates a close relationship. It was appreciated that the catabolic rate of the existing body pool, during depletion, as demonstrated in column "c" of Table 8 was not a true reflection of the metabolic requirements of humans and, therefore Hodges and Canham (22) presented a calculated average daily need which provided an amount to supply the metabolic needs and maintain the body pool. Unfortunately the data upon which the calculated average daily need was based were incompletely analyzed. Hence the calculated average daily need presented in reference 22 is only partially correct. In column "d" of Table 8 the calculated average daily need based upon completely analyzed data are presented. It can be seen that when utilization rates are expressed in that fashion that there is a close correlation between fat free mass and the calculated average daily need. The observations made by Baker et al (3) were in normal adult humans receiving a normal dietary intake. It appears that during vitamin C deficiency and repletion, the relationship of the fat free mass to body pool and utilization is not as well defined but, in general, they are related.

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22. Hodges, R.E. and J.E. Canham. US Government Printing Office, Wash, D.C., Pub No 916.086, 1971.

Recently Kallner et al (23) have suggested that the technique used in this study for estimating the vitamin C body pool size overestimates the actual pool size. They studied non-smoking males who were maintained on a diet of normal foods assumed to be low in vitamin C but whose vitamin C content apparently was not controlled. The subjects were divided into groups with each group provided a fixed daily ascorbic acid supplement ranging from 30 to 180 mg per day. In these subjects ascorbic acid pool size was determined and expressed in terms of mg per kg of body weight. Unfortunately, Kallner et al (23) apparently did not measure any of the body compartments nor were the weights of the subjects provided in order that the total pools could be calculated. The body pool did vary from 11.4 to 21.6 mg per kg of body weight with a mean of 16.8 (23). The pool sizes estimated on our subjects expressed in Kallner's mg per kg of body weight would range from 17.2 to 31.1 mg per kg of body weight with a mean of 24.

CONCLUSION

Experimental scurvy was induced in five adults males, 26 to 52 years of age. Body compartment measurements during recovery were derived from body density by water displacement, total body water from deuterium dilution and total body potassium from whole body counting of ^{40}K. The first body composition measurements were accomplished after measured amounts of vitamin C had been administered daily for 18, 24, or 31 days. A second measurement was made approximately 17 weeks later. It can be concluded that:

1. Attempts to maintain constant body weight failed because of variability in the acceptability of the diet provided during repletion and the limited opportunity for energy expenditure.

2. During repletion body fat exhibited the greatest mean body compartmental change.

3. In early repletion significant negative correlation existed between body pool, plasma or whole blood ascorbate and the dry protein mass.

4. All of the techniques used to estimate the size of the dry protein compartment did not detect evidence of impaired protein utilization during recovery from vitamin C deficiency but data in Scurvy II did not suggest the impaired protein utilization noted in Scurvy I.

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LIST OF TABLES

TABLE 1. Body composition of previously scorbutic subjects after 18 to 31 days and 17 to 20 weeks repletion.

	After 18-31 days of (early) Repletion		After 17-20 weeks of (late) Repletion	
Density g/ml	1.051 ± 0.0.4*		1.038 ± 0.013**	
	kg	% body weight	kg	% body weight
Body weight	72.34 ± 6.96		77.10 ± 11.28	
Water (derived)	39.99 ± 5.37	55.3	39.90 ± 5.98	51.8
Fat	17.56 ± 4.84	24.3	22.45 ± 6.52**	29.1
Dry protein	11.06 ± 1.49	15.3	11.03 ± 1.65	14.3
Mineral	3.72 ± 0.50	5.1	3.71 ± 0.56	4.8
Water (observed D_2O dilution)	43.52 ± 5.01	60.2	43.96 ± 5.10	57.0

* Mean and S.D. of five subjects.
** Significantly different from early repletion ($p<0.05$).

TABLE 2. Individual data for changes (kg) in body composition during ascorbic acid repletion.

Subject	Body Weight	Water	Fat	Protein	Mineral
H	7.97	0.89	6.76	0.14	0.08
P	8.23	0.86	7.03	0.24	0.08
S	8.32	0.03	8.28	-0.04	-0.01
M	-1.06	-1.75	1.34	-0.48	-0.16
R	0.34	-0.51	1.04	-0.03	-0.01
Mean	4.76	-0.09	4.89	-0.03	-0.01

TABLE 3. Comparison of estimates of dry protein mass (kg) as derived from total body water, body density and potassium-40 whole body counting.

	DRY PROTEIN (kg)		
	Body Water	Body Density	Whole Body Counting
		EARLY REPLETION	
H	13.85	13.13	13.50
P	12.19	10.82	9.52
S	12.53	11.56	9.78
M	11.34	10.78	10.03
R	10.14	9.02	8.44
Mean	12.01 ± 1.38	11.06 ± 1.49	10.25 ± 1.91
		LONG TERM REPLETION	
H	13.80	13.37	13.60
P	12.58	11.06	11.62
S	12.77	11.52	12.16
M	11.37	10.30	10.86
R	10.14	8.88	9.13
Mean	12.13 ± 1.41	11.03 ± 1.65	11.47* ± 1.65

* Significant change (P<0.05) between early and late repletion.

TABLE 4. Ascorbic acid levels (mg) upon specified repletion day.

	Day	Total repletion ascorbic intake, (mg) by day of measurement	Body pool (mg)	Plasma mg/dl	Whole Blood mg/dl
			Early Repletion		
H	24	134.5	107.5	0.11	0.36
P	24	134.5	129.5	0.13	0.42
S	31	1229.5	863.0	0.18	0.50
M	31	4014.5	2026.0	0.20	0.30
R	18	1197.0	830.5	0.18	0.62
			Late Repletion		
H	143	>4000	1500.0+	1.84	2.29
P	143	>4000	1500.0+	1.69	2.05
S	150	>4000	1500.0+	1.86	2.01
M	150	>4000	2026.0+	1.33	1.52
R	137	>4000	1500.0+	2.06	2.25

TABLE 5. Correlation coefficients of body fat and dry protein mass to ascorbic acid.

	BODY FAT			DRY PROTEIN		
	D_2O	Density	^{40}K	D_2O	Density	^{40}K
				Early Repletion		
Vitamin C						
Body Pool	-0.127	-0.291	-0.831	-0.518	-0.355	-0.354
Plasma	-0.016	-0.102	-0.786	-0.718	-0.611	-0.669
Whole Blood	0.021	0.210	-0.085	-0.526	-0.618	-0.595
				Late Repletion		
Body Pool	-	-	-	-	-	-
Plasma	0.107	0.084	0.317	-0.067	-0.054	-0.117
Whole Blood	0.277	0.164	0.312	0.220	0.246	0.168

TABLE 6. Change in selected anthropometry early vs long term repletion of ascorbic acid.*

Diameter	Early Repletion cm	Late Repletion cm	% change
Bideltoid	47.19 ± 1.37	48.00 ± 3.36	1.7
Bihumeral	48.55 ± 2.10	49.94 ± 3.79	2.9
Bi iliac	28.57 ± 1.65	29.28 ± 2.67	2.5
Anterior - Posterior chest	22.02 ± 1.74	23.32 ± 1.63	5.9
Lateral chest	31.61 ± 1.61	32.62 ± 2.81	3.2
Circumferences			
Forearm	26.98 ± 1.67	27.80 ± 2.13	3.0
Biceps	29.84 ± 0.71	30.84 ± 2.64	3.4
Calf	35.30 ± 1.98	37.66 ± 2.79**	6.7
Waist	90.58 ± 5.16	91.78 ± 8.70	1.3
Buttocks	97.16 ± 3.42	99.96 ± 4.93	2.9
Skinfolds	mm	mm	
Triceps			
Right	17.0 ± 6.5	12.4 ± 4.17	27.1
Left	16.4 ± 6.4	11.0 ± 4.14	32.9
Scapula			
Right	18.4 ± 6.5	16.9 ± 10.1	8.2
Left	18.7 ± 6.0	16.9 ± 7.5	9.6

* =Mean ± standard deviation.
** =Significantly ($P < 0.05$) different than data obtained in early repletion.

TABLE 7. Body weight (kg) of the volunteers from the end of the control period until late repletion.

Subject	End of Control Period	Wgt end of Depletion	Wgt at time of first body comp measurement	Late repletion
H	84.1	87.0	77.41	85.38
P	85.2	81.1	78.99	87.22
S	79.7	76.0	74.52	82.84
M	67.3	71.5	68.76	67.70
R	64.5	66.2	62.03	62.38
Mean	76.2	76.4	72.34	77.10

TABLE 8. Comparison of fat free mass with body pool and utilization rates of ascorbate.

Subject	a Fat free mass, Kg. Early repletion	b Estimated Initial body pool of ascorbate, mg	c Rate of catabolism of existing body pool %/day	d Calculated average daily need (mg/day)[2]
H	65.03 (66.24)[1]	2570	4.1	26.0
P	53.55 (54.75)	1625	3.5	21.4
S	57.28 (57.32)	1920	2.6	22.9
M	53.39 (50.99)	2140	3.2	21.4
R	44.67 (43.97)	1125	2.8	17.9
	54.78 ± 7.36	1876 ± 543	3.2 ± 0.6	21.9 ± 2.9

1 - Fat free mass, late repletion in parens.
2 - To supply metabolic needs and maintain pool(s).

	a vs b	a vs c	a vs d
Correlation coefficient (R)	0.922	0.651	0.996
Coefficient of determination (r^2) for curve fitting:			
Linear curve	0.8505	0.424	0.991
Parabolic curve	0.8505	0.688	0.992

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